PHYLOGEOGRAPHIC PATTERNS AMONG THE FRESHWATER MUSSEL *ELLIPTIO* LANCEOLATE SPECIES COMPLEX

A Thesis
by
HANS ROBERT LOHMEYER

Submitted to the School of Graduate Studies
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

May 2020
Department of Biology

PHYLOGEOGRAPHIC PATTERNS AMONG THE FRESHWATERMUSSEL

A Thesis
by
HANS ROBERT LOHMEYER
May 2020

APPROVED BY:

Michael M. Gangloff Ph.D.
Chairperson, Thesis Committee

Nathan A. Johnson, Ph.D.
Member, Thesis Committee

Matt C. Estep, Ph.D.
Member, Thesis Committee

Lynn M. Siefferman, Ph.D.
Member, Thesis Committee

Zack E. Murrell, Ph.D.
Chairperson, Department of Biology

Mike McKenzie, Ph.D.
Dean, Cratis D. Williams School of Graduate Studies

Abstract

PHYLOGEOGRAPHIC PATTERNS AMONG THE FRESHWATER MUSSEL *ELLIPTIO* LANCEOLATE SPECIES COMPLEX

Hans R. Lohmeyer
B.S., University of North Carolina at Asheville
M.S., Appalachian State University

Chairperson: Dr. Michael M. Gangloff

Integration of molecular, morphological and biogeographic data improves our ability to elucidate species boundaries and phylogenetic relationships. This approach also benefits field research by improving the ability of biologists to recognize species using comparisons of phenotypic attributes. The extreme morphological variation among *Elliptio* species has led to the proliferation of species names and generated a long-running debate about the phylogenetic structure within this genus. Although earlier studies have considered *Elliptio* to be comprised of three species complexes; *E. complanata*, *E. icterina* and *E. lanceolata*, current species lists recognize 30 *Elliptio* taxa including 7 taxa in the lanceolate group. Within the lanceolate group, *E. lanceolata* is currently listed as federally threatened under the Endangered Species Act. I examined phylogenetic relationships among seven-species within the lanceolate *Elliptio* complex from 20 Atlantic Slope river basins using both mitochondrial (COI and NDI) and nuclear (ITS-1 and 28S) DNA sequences. I constructed haplotype networks to examine species boundaries and biogeographic trends of gene exchange and to guide my single gene and multi gene Maximum likelihood and Bayesian phylogenetic

analyses. My data revealed the existence of three taxa in the lanceolate *Elliptio* complex. *E. lanceolata* was recovered as a monotypic and highly divergent from the core non-lance *Elliptio* group (*E. complanata* and related taxa). I also found support for two morphologically distinct and genetically divergent lineages, a northern *E. fisheriana* and a southern *E. angustata* lance clade that are more closely related to the core *Elliptio* group than the *E. lanceolata* taxa. Future steps are to revise taxonomy and provide guidance to resource managers tasked with managing this imperiled group of organisms.

Acknowledgments

First and foremost, I would like to thank my advisor, Dr. Michael Gangloff. This project would not have been possible without your advisement and guidance. I am truly grateful for allowing me to join the ACRL lab and pushing me to expand my conservation experience and understanding. I would also like to thank each of my committee members. Dr. Nathan Johnson, I am thankful for the opportunity that you have provided and the patience you have shown me while I worked through the development and completion of my thesis project. Dr. Matt Estep, I will never forget the guidance you provided me and the kindness of you sitting down with me and helping me understand the foundational principles of molecular ecology. Dr. Lynn Sifferman, your input into my presentations and presenting style helped me simplify my thoughts and ideas on a project and field of research that has tested me in doing so.

I would also like to thank the North Carolina Wildlife Resources Commission Aquatic Wildlife Diversity Program for funding and specimen sampling. To all the members of the ACRL lab, your collective effect on both my project and personal experience, helped me reach the finish line with some of my sanity still intact. Victoria Fowler, I am extremely thankful for you taking me in, showing me the phylogenetic ropes when I first joined the lab and continuously hosting break times to discuss our projects. Vincent Santini, your friendship and willingness to always include me in social and field sampling events provided me with much needed experience in mussel identification and weird jam band concerts. Chantelle

Rondel, the determination you showed on your project and the kindness you showed within the lab was always inspirational to me and I look forward to working with you in the future. Sam Fritz, Freddy Ortega and Amber Olson, each one of you allowed me the opportunity to decompress from the craziness of my project with your ridiculous memes and weird internet videos. Jokes aside, you guys were always in lab and willing to bounce off ideas and discuss my project and I am truly appreciative of your openness. Michael Thompson, your level headedness and logical train of thought has provided me with a much-needed perspective to help complete my project. I would also like to thank the newest arrival Brandon Williams, for some extra help working through phylogenetic software. Sequence analysis can sometimes be a lonely and frustrating journey and I appreciate you helping me through a portion of it.

Finally, I would like to thank my wife, Cassandra Lohmeyer for her constant support throughout these two years. You provided me with the confidence and inspiration I needed to complete this project, while also reminding me to take breaks when they were needed.

Table of Contents

List of Tables

List of Figures

Foreword

This research will be submitted to the peer-reviewed journal, *Molecular Phylogenetics and Evolution*. It has been formatted to fit the requirements for that journal.

Introduction

Biodiversity is a fundamental component of ecosystem function and productivity (Duffy et al., 2017; Van der Plas, 2019). Globally freshwater biodiversity is declining rapidly because of human disturbances (Dudgeon et al., 2006; Tickner et al., 2020). This loss of freshwater diversity leads to dramatic declines in ecosystem health and ecosystem services that have cascading effects resulting in harmful conditions for human health (Chivian, 2002). Freshwater bivalves play an important role in the mitigation of damage to our freshwater ecosystems. Bivalves provide multiple ecological services such as pollution removal and substrate stabilization (Vaughn and Hakenkamp, 2001). Currently North America contains the highest number of endemic freshwater mussel taxa in the world, with ~300 described species. To date, 35 freshwater bivalve species have been declared extinct and 75% of species within the Family Unionidae are imperiled (Haag and Williams, 2013). Conservation and management efforts for priority mussel taxa have been inhibited by a lack of defined taxonomic and phylogenetic relationships due to the largely understudied effects of phenotypic plasticity on shell morphology. Integration of molecular, morphological and biogeographical data can help better elucidate species boundaries and phylogenetic relationships in these taxa and provide important insights into aquatic biodiversity (Campbell et al., 2008; Smith et al., 2018).

Prior to the advent of protein- or DNA-based genetic research, freshwater mussel taxonomy largely relied on shell morphology to identify species in the field or diagnose new species (Davis, 1984; Johnson, 1970) However, in organisms that exhibit extensive phenotypic plasticity, species diagnoses inferred from phenotype alone can substantially over- estimate taxonomic richness. The extreme morphological variation observed among freshwater mussel

species has led to a large number of nominal species descriptions over the past 200+ years and it has been the job of field and museum-based zoologists and, more recently, geneticists to assess the taxonomic and geographic boundaries of these taxa (Campbell et al., 2008; Johnson, 1970; Williams et al., 2014). The genus Elliptio provides an archetypal example of this process. Elliptio was first designated as a subgenus (of Unio) comprised of 12 species by Rafinesque (1819). Ortmann (1912) elevated *Elliptio* to the genus level and placed eight taxa within this group (*Elliptio crassidens*, *Ellipto beadleianus*, *Elliptio spinosus*, *Elliptio complanatus*, *Elliptio jayensis*, *Elliptio productus*, *Elliptio gibbosus*, and *Elliptio popei*. Later malacologists (e.g., Johnson, 1970) appeared to recognize that phenotypic plasticity was widespread in this group and considered *Elliptio* to be comprised of three species complexes (*E. complanata*, *E. icterina* and *E. lanceolata*) with the highest number of taxa presumed to occur in rivers draining the southeastern Atlantic Slope of North America (Johnson, 1970). However, the number of species recognized in *Elliptio* has ranged from 13 (Johnson, 1970) to 48 (Turgeon et al., 1998) in recent accounts.

Davis et al. (1981) were among the first to use allozymes to examine genetic differences within and among freshwater mussel taxa. They showed a distinction between mussels in the *E. lanceolata* complex and those in the *E. complanata* complex (Davis et al., 1981). Later, Davis (1984) hypothesized that many *Elliptio* species were very closely related, despite extensive variation in shell morphology and potentially recent radiation. A lanceolate *Elliptio* phylogeny, was never published from this work. More recently, the widespread availability and use of genetic analysis have added some clarity to the deeper phylogenetic patterns within *Elliptio* and these patterns are reflected in recent taxonomic changes. For example, *Eurynaia dilatata* and

Parvaspina steinstansana were formerly classified as *Elliptio* but were re-assigned to other genera based on genetic analyses (Campbell and Lydeard, 2012; Perkins et al., 2017). Inoue et al. (2018) published a broad scale examination of phylogenetic patterns with the *Pleurobemini* and suggested *E. lanceolata* may not be a 'true' *Elliptio*. Currently *Elliptio* is believed to comprise 30 taxa (Williams et al., 2017) but there has not yet been a comprehensive attempt to examine genetic data across this group and most taxa remain poorly-sampled.

Elliptio taxa that are considered to be part of the *E. lanceolata* complex (or more accurately, the Lanceolate *Elliptio* complex or LEC hereafter) are characterized by elongate, blade-like shells that are often more than twice as long as high and have dorsal and ventral margins that are roughly parallel (Johnson, 1970). Additionally, shells often exhibit a high degree of lateral compression. Seven LEC taxa (*E. lanceolata, E. shepardiana, E. fisheriana, E. ahenea, E. angustata, E. producta and E. folliculata*) were considered to be valid taxa by the most recent comprehensive list of freshwater mussel taxa (Williams et al., 2017). Of these, *E. lanceolata* (Yellow Lance) is federally listed as threatened under the U.S. Federal Endangered Species Act and *E. ahenea* (Southern Lance) and *E. shepardiana* (Altamaha Lance) are listed as near threatened (Bogan, 1996; USFWS, 2016).

The purpose of this study is twofold. First, I elucidate taxonomic boundaries among LEC taxa using mtDNA and nDNA haplotype networks. Second, I examine the phylogenetic placement of LEC taxa within *Elliptio* and the tribe Pleurobemini using a multi-gene dataset and assessed whether this group may comprise one or more distinct genera as has been speculated by earlier researchers (Bogan, 2009; Davis, 1984; Inoue et al., 2018). These data will provide both a more robust understanding of species diversity and evolutionary relationships within a

widespread, ecologically-important and potentially at-risk group of freshwater mussels in a region of the world that has experienced recent widespread and unexplained declines. Genetic data may also help inform both habitat conservation and restoration as well as captive propagation and translocation programs designed to help protect and recover at-risk mussel taxa.

Materials and Methods:

Taxonomic Coverage

I examined material from all seven LEC taxa recognized by Williams et al. (2017). I obtained tissue samples from museum specimens or field collected between 2007 and 2019 from 25 populations across six states (FL, GA, SC, VA, NC and MD) within the southeastern United States. Tissue samples were collected non-lethally from all *E. lanceolata* and *E. ahenea* individuals using sterile buccal swabs (Isohelix SK-1 swabs, Boca Scientific Inc., Boca Raton, FL) and frozen at -20 C until extraction. *Elliptio lanceolata* (n=24) were collected from three populations in NC (Fishing Creek, Tar River, and Swift Creek), one population in VA (Carter Run) and one population in MD (Hawlings River*). Elliptio ahenea* (n=17) from seven populations in FL (Fisheating Creek, Arbuckle Creek, Redwater Lake, St. Marys River, Suwannee River, Unnamed tributary to the New River and Ocklawaha River (Table 1.) *Elliptio fisheriana* (n=29) were collected from two population in SC (Santee River and Congaree River), four populations in NC (Big Creek, Lake Waccamaw, Dan River and Roanoke River) and two populations in MD (Zekiah Swamp Run, and Unicorn Branch).

Elliptio angustata (n=4) were collected from two populations in SC (Catawba River and Congaree rivers) and *Elliptio shepardiana* (n=5) were collected from two populations in GA

(Altamaha and Ohoopee rivers). *Elliptio folliculata* (n=5) from one population in NC (Lake Waccamaw)(Table 1).

All non-listed specimens were collected by hand and vouchered. Adductor tissue was clipped and placed in 95% EtOH and the animals were vouchered in the Appalachian State University Zoological Collections in Boone, North Carolina. These collections were supplemented with materials collected and sequenced by collaborators with USGS and Florida Fish and Wildlife Commission (N. Johnson and J. Williams respectively) or biologists with the North Carolina Wildlife Resources Commission's (NCWRC) Freshwater Nongame Diversity Program. A total of 27 sequences from outgroup taxa were included in genetic analyses (Table 2). A small number of sequences were also obtained from Gen Bank (Table 3).

Phylogenetic Sequencing and Analysis

I isolated and purified total genomic DNA was isolated and using Qiagen DNeasy Blood and Tissue Kit (Qiagen Sciences Inc., Valencia CA) following manufacturer protocols. I determined DNA concentration and quality using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham MA) and stored long term at -20 C at Appalachian State University (Boone, NC) facilities. Regions of the mtDNA cytochrome oxidase subunit I (COI) and NADH dehydrogenase subunit I (NDI) genes as well as nDNA internal transcribed spacer region 1 (ITS-1) and 28s ribosomal RNA (28s) were amplified for all available LEC specimens.

I used COI primers developed by Campbell et al. (2005) and based on the universal Folmer et al. (1994) primer set as well as NDI primers adapted from Serb et al. (2003) by Fagundo (unpub.2016) (Table 4.). I ran PCR sequences on an Eppendorf Mastercycler. I used the

following conditions in PCR reactions: 12.5 µL GoTaq. Green Master Mix 2X (Promega Corporation, Madison, WI), 0.4 µL each primer (0.5 µM), 10–50 ng/µL DNA template, and nuclease-free water to a final volume of 25 µL. PCR amplifications for ITS-1 were performed following conditions outlined in King et al. (1999) and amplification for 28s were performed following conditions outlined in Therriault et al. (2004) (Table 4). I visually inspected PCR products on a 1% agarose gel stained with ethidium bromide and sent successful reactions to Retrogen Inc. (San Diego, CA) for sequencing.

I compiled, edited and aligned sequences in Geneious R7 (Biomatters Ltd., Aukland, New Zealand) using MAFFTv7.299 (Katoh and Standley, 2013) following default parameters, I translated and aligned protein coding mitochondrial loci COI to check for absence of gaps stop codons and testing for homologous characters. I calculated uncorrected p distances in MEGA v7 (Kumar et al., 2016) to showcase evolutionary divergence within the LEC, the *Elliptio* genus and between two taxa that were recently re-assigned from *Elliptio*, *Eurynaia dilatata* and *Parvaspina steinstansana* (Campbell and Lydeard, 2012; Perkins et al., 2017). I predetermined the LEC groupings based on concatenated Maximum likelihood (ML) and Bayesian inference topologies (BI). Estimated number of haplotypes, and mean nucleotide diversity (π) were calculated for the LEC dataset using DNASP v6.12.03 (Rozas et al., 2017). I then generated a haplotype network individually for COI, NDI and ITS-1 using a TCS network and coded each network by species (Clement et al., 2002) (Fig 1, Fig 2 and Fig 3.) I also generated a TCS network for my CO1 dataset and coded it by drainage (Fig 4).

I implemented jModelTest 2.1.9 (Darriba et al., 2012) to select the best fit model of nucleotide substitution and formed a partition file to partition separate substitution models for

multiloci analysis. Two selection criteria (Akaike Information Criterion (AICc) with finite population correction and Bayesian Information Criterion (BIC)) identified the best-fit substitution models for Bayesian inference (BI) and Maximum Likelihood analyses (ML) within a 95% confidence interval. I analyzed concatenated mtDNA, nDNA and 4 loci datasets using maximum likelihood in the program IQ-tree (Nguyen et al., 2015) and Bayesian inference in the program Mr. Bayes v 3.2.7 (Ronquist et al., 2012) using the CIPRES Science Gateway (Miller et al., 2010). mtDNA analysis included a total of 132 sequences for both COI and NDI loci. I conducted ML analysis using 1000 tree searches using 2000 Ultrafast bootstrapping method (Hoang et al. 2018). I computed Mr. Bayes analyses using 2 runs of 7 chains for 1x 107 generations sampling every 1000 trees. To determine the proper burn-in value, I analyzed log likelihood scores for each sampling point using Tracer v 1.7.1 (Rambaut et al., 2018). I visualized and edited the phylogenetic trees produced were visualized and edited in FigTree v 1.4.4 (Rambaut et al., 2018).

Results

Taxon, Character Sampling and Haplotype Networks

I sampled and sequenced 100 LEC specimens. The final concatenated mtDNA (COI and NDI) dataset was made up of 132 total specimens and sequences (100 LEC specimens and 32 outgroup specimens). The COI data set consisted of ~659 base pairs (bp) and the NDI dataset was ~901 bp with a total concatenated mtDNA alignment of ~1,558 bp. The ITS-1 and 28s consisted of 108 specimens with the alignment including a total of 1330 base pairs with ITS-1 consisting of ~576 base pairs including an avg. of 16.3% gaps and 28s with ~755 and 1.0% avg. gap content. The 4 loci alignment (COI, NDI, ITS1 and 28s) consisted of a total of 2,888 bp with a gap percentage

averaging 7.0%. My mtDNA (COI and NDI) alignment (n=100 LEC specimens) contained 56 unique haplotypes.

Analyses of mtDNA haplotype networks revealed a high degree of haplotype sharing among five nominal LEC taxa: *E.ahenea*, *E.angustata*, *E. producta*, *E. shepardiana*, and *E. folliculata.* These taxa exhibited an average uncorrected pairwise genetic distance of ~.47% (CO1) ~.53% (ND1) and ~.68% (ITS1) (Table 5-Table 7). The Southern LEC clade (*angustata* is the oldest name and appears to have priority) is distinct and separated by at least 64 mutations from a northern LEC clade for which the best name appears to be *E. fisheriana*. The northern LEC clade shows no evidence for haplotype sharing with other LEC taxa (Fig. 1 and Fig. 2). However, there does appear to be some geographic structuring in this clade (Fig. 4) as populations in three different geographic ranges (Northern Atlantic slope, Southern Atlantic Slope and Roanoke (Dan River) exhibit somewhat divergent interspecific pairwise distances that averaged 2.7% different from one another (Table 7). *Elliptio lanceolata* is genetically distinct from other LEC taxa and was separated by at least 120 mutations from the core *Elliptio* group. Mean pairwise distances observed between *E. angustata* (avg. 8.5% (CO1) and 11.3% (ND1) and *E. fisheriana* (avg.8.2% (CO1) and 9.8% (ND1) (Table 5 and Table 6) suggest that these taxa do not belong to the same genera. In contrast, genetic distances within *E. lanceolata* were relatively low and although populations in the Potomac River did not share haplotypes with populations in drainages further south, overall differences were well within the range of intra-specific divergence levels (mean = 2.5% uncorrected pairwise distance**,** Table 8). Finally, the ITS-1 haplotype network had a total of 15 unique haplotypes with a low nucleotide diversity value of n= 0.021 (Fig. 3).

Phylogenetic Analyses

I selected results for best fit substitution models for BIC and AICc based on which model included the highest value of log likelihood. Within the concatenated mtDNA dataset BIC and AICc models chosen were General time reversible model (GTR) including Empirical base frequencies (+F) proportions of invariable sites (+I) and a discrete Gamma model with four categories (+G4), nDNA (BIC:Hasegawa, Kishino and Yano model (HKY+F+I+G4), AICc: GTR+F+I+G4) and 4 loci alignment (BIC and AICc: GTR+F+I+G4) all within a 95% confidence interval.

The phylogenetic reconstruction based on my concatenated four gene alignment is presented for BI in (Fig. 5) and ML in (Fig. 6). Both BI and ML analyses interpreted three separate clades within the LEC taxa. LEC taxa comprise a polyphyletic clade with *Elliptio lanceolata* grouping very distantly from other LEC taxa and this taxon appears to form a monophyletic clade with *Parvaspina*. This relationship is statistically well-supported (bootstrap support 80%, BPP = 97) and was also returned in concatenated mtDNA BI and ML analyses (bootstrap support 76%, BPP = 99, Fig. 7 and Fig. 8). Analyses of the concatenated nDNA (ITS1 and 28s) alignment found that although *E. lanceolata* formed a clade sister to other LEC taxa, this grouping was not well-supported (bootstrap support 64%, BPP = 53, Fig. 9, Fig. 10). The remaining six LEC taxa form a well-supported monotypic clade that included the 'core' *Elliptio* taxa *E. crassidens* (the type species for the genus *Elliptio*) in both the 2 gene mtDNA analysis (bootstrap values of 100 for BI analyses and 93 for ML) and the 4 gene analysis (BI bootstrap value = 100, ML bootstrap value = 94) Analysis of nDNA data did not support these groupings

with LEC taxa grouping sister to *E. dilatata* but not *E. crassidens*, a highly incongruent and poorly supported topology.

Discussion

These data reveal that the lanceolate *Elliptio* complex is a paraphyletic group comprised of two genera; a lineage that is sister to and likely belongs within *Elliptio sensu strictu* and a highly divergent lineage comprised of *E. lanceolata* that appears to be sister to *Parvaspina* in the majority of well-supported topologies. Examination of data from the remaining six lanceolate taxa (*E.angustata*, *E. ahenea*, *E. fisheriana*, *E. folliculata*, *E. producta* and *E. shepardiana*) reveal the existence of two distinct and more closely-related clades that are sister to (and likely belong within) the core *Elliptio* clade (e.g., *E. crassidens* in Gulf Slope and *E. complanata* in Atlantic Slope drainages). Data support taxonomic synonomyzation of *E. ahenea*, *E. angustata*, *E. folliculata*, *E. producta*, and *E. shepardiana* with *E. angustata* having taxonomic priority within this clade of southern Atlantic slope lances. A second clade is comprised of individuals from mid- and northern Atlantic Slope drainages and includes topotypic *E. fisherina* and I therefore reserve *fisheriana* for the Northern lance lineage. These results substantially change our understanding of diversity within *Elliptio* and my taxonomic recommendations would reduce the number of lanceolate *Elliptio* taxa from seven to two.

Elliptio lanceolata

Three lines of evidence, phylogenetic reconstructions, haplotype networks and pairwise distance matrices, all provide strong support for the hypothesis that *E. lanceolata* represents a distinct

evolutionary lineage within the Pleurobemini and that a lanceolate shell morphology represents a convergent morphological characteristic. *Elliptio lanceolata* was distant from *Elliptio sensu strictu* in all BI and ML phylogenies that included mtDNA data. These results suggest that *E. lanceolata* should likely be reassigned to a monotypic genus. Although, *E. lanceolata* was found to be sister to *Parvaspina* in both mtDNA and 4 loci phylogenies, the pairwise distance matrix for the mtDNA revealed high levels of genetic divergence (>10%) that are consistent with inter-generic differences found among other *Pleurobemini*.

Results of my analysis are congruent with prior molecular analyses. One of the first genetic studies (Davis et al., 1981) electrophoretic techniques to examine *E. shepardiana*, *E. folliculata* and *E. fisherina* were included in separate lineages from *E. lanceolata*. A year later Moore et al. (1982) quantified significant morphometric differences between *E. angustata* and *E. lanceolata*. In an unpublished report for the Virginia Department of Transportation, Bogan et al. (2009) also suggested that *E. lanceolata* was a distinct taxon within the lanceolate group and that a new genus may be needed to accurately depict the evolutionary distinctiveness of *E. lanceolata*. Inoue et al. (2018) suggested that *E. lanceolata* was evolutionarily distant from other *Elliptio* taxa and appeared to clade more closely with *Parvaspina*.

Because *E. lanceolata* is currently listed as federally-threatened under the Endangered Species Act these findings may have significant conservation implications and may influence future management decisions for this species. haplotype networks revealed relatively low levels of range-wide genetic variability which what might be expected from a taxon that occurs across such a broad geographic range. However, the low levels of genetic diversity observed may also

reflect the imperiled conservation status of the Yellow lances (USFWS, 2016) and the fact that many of the sampled populations were small and or highly fragmented and may be in danger of inbreeding depression due to reduced gene flow. Additionally, my results suggest that *lanceolata sensu strictu* is restricted to mid-Atlantic drainages extending from the Neuse north to the Patuxent/Potomac drainages. Historical taxonomic uncertainty makes assessing changes to historical distributions and conservation status challenging. These data suggest that records of *E. lanceolata* outside of this range should be regarded with skepticism although physical examination of vouchered materials is sufficient to alleviate any uncertainty.

Elliptio angustata and E. fisheriana

Both my concatenated mtDNA and 4 loci phylogenies suggest that the other six LEC taxa form a clade that is distinct from *E. lanceolata* but that is closely-related, and likely sister, to the Core *Elliptio* clade. haplotype networks highlight possible evidence of geographic structuring within *E. fisheriana* that are statistically supported with genetic distances from pairwise matrices but very little evidence of genetic structuring within taxa in Southern Atlantic Slope Drainages.

Results of haplotype network and pairwise difference analyses both provide support for synonymizing the five southern LEC taxa (*E. ahenea*, *E. angustata, E. folliculata*, *E. producta* and *E. shepardiana*) into a single taxon, *E. angustata*. However, the Southern *angustata* clade shows little to no geographic structuring among the five taxa that are included. Interestingly, based on my dataset, this clade geographically ranges from Florida (St. John River Drainage) to North Carolina (Neuse Drainage) with specimen also included in the upper Pee Dee drainage of North Carolina. Large amounts of morphological variation within *E. angustata* has likely fueled taxonomic confusion. The influence of abiotic factors on mussel shell morphology has been

well-studied and authors beginning with Ortmann have noted that shells are often more elongate and thin shelled in headwater streams (Bailey and Green, 1987). Despite this, phenotypic plasticity of *Elliptio* is daunting and, as a result, is largely unstudied beyond gestalt-based comparisons of shell morphology among and within taxa (Williams et al., 2017). *Elliptio angustata* includes five nominal taxa (*E. ahenea*, *E. angustata*, *E. folliculata*, *E. producta* and *E. shepardiana*) that, despite exhibiting large amounts of shell shape variability exhibited little to no genetic structuring among the morphological types examined.

My study supports, in part, the synonomization of lanceolate Elliptio taxa as suggested by Johnson (1970). However, Johnson (1970) did not include *E. ahenea* and *E. shepardiana* as synonyms of *E. lanceolata*. Later, Davis (1984) computed pairwise matrices of genetic distance based on allozymes and suggested that *E. folliculata* and *E. shepardiana* were closely related. Davis (1984) also found that *E. producta* and *E. fisheriana* were synonomous, a result not supported by mtDNA data. Prior studies did not, however, assess whether *E. angustata*, *E. fisheriana* or other lance taxa are sister to the core *Elliptio* clade. Results of my phylogenetic reconstructions as well as haplotype networks and pairwise distance values for both CO1 and ND1 loci (generally <6% divergent) all suggest that this group should remain within the genus *Elliptio*.

Some forms of the northern lance may have been mis-identified in past studies as dark and lightly rayed *Elliptio lanceolata*. For example, *E. fisheriana* was considered to be an ecophenotype of *E. lanceolata* by Johnson (1970). However, Moore et al. (1982) examined morphometric and soft anatomy differences between *E. lanceolata* and *E. angustata* and found that the two taxa were highly divergent in multivariate space based on 14 different

morphological measurements. Numerous specimens examined by Bogan et al. (2009) were also initially identified in the field as *E. lanceolata* but later shown by genetic analyses to be *E. fisheriana*. This study and my data both revealed deep genetic differences between *E. fisheriana* and *E. lanceolata* that were comparable to those between *E. angustata (*and all synonymous taxa) and *E. lanceolata*. Taken together these results suggest that it is highly unlikely that the *E. angustata-fisheriana* clade is closely related to *E. lanceolata*. However, analyses of the nDNA loci found that the *E. angustata*/*E. fisheriana* clade is sister to *Parvaspina*, these data may reflect incomplete lineage sorting in mtDNA lineages or, more parsimoniously, historical relationships between ancient evolutionary lineages within the *Pleurobemini*. Examination of CO1 and ND1 haplotype networks revealed that the Northern lance (i.e.,*E. fisheriana*) clade can be sub-divided into 3 major demes that correspond to different geographic regions across its range. The range of the northern deme extends from the Chester Drainage in Maryland south to the lower Roanoke Drainage in North Carolina and the southern deme ranged from Lake Waccamaw in North Carolina south to the Santee River in South Carolina. The third and final geographically-isolated deme is found exclusively within the upper Dan River Drainage in North Carolina. This deme is substantially different (>1.5%) from the northern deme including an individual found in the lower Roanoke River. This genetic distance may reflect reproductive isolation of this lineage in the geologically-ancient (Mesozoic) Roanoke River headwaters (which include the Dan River, Horton et al., 1991). Similar examples of genetic isolation of endemic mussels are well-documented in the similarly-ancient headwaters of the Mobile and Tennessee-Cumberland drainages (Lane et al., 2019; Roe et al., 2001; Smith et al., 2019).

Taxonomic and Management Implications

My data reveal a remarkable degree of morphological convergence exhibited by taxa from across a substantial evolutionary distance. Ortmann (1919) separated *Unio fisherianus* from *Unio lanceolata* on the basis that *U. fisherianus* was slightly more elongated with a greater taper to the posterior part of the shell. Davis et al. (1981) interpreted deep genetic differences between these taxa as evidence that the lanceolate shape appears to have evolved concurrently in multiple lineages. Wolfe (1984) noted that lance taxa were similar in appearance but that soft tissues lacked other distinguishing traits.

However, my data also reveal that both *E. angustata* and *E. fisheriana* co-occur across a range that extends at least from the Santee Basin in South Carolina north to the Tar-Pamlico Basin in North Carolina. This appears consistent with the extent of geographic range overlap proposed by Bogan and Alderman (2006) and Bogan and Ashton (2016). The variability of field identifications of specimens used in this study reflects the difficulties associated with separating these taxa based on morphological traits. Lance-like shells are not exclusive to *Elliptio* but are common across a range of other freshwater mussel genera in both North American (e.g., *Lampsilis*, *Ligumia*, *Villosa*) and Asian (e.g., *Lanceolaria*) Unionidae as well as in other pearly-mussel lineages including Hyriidae (e.g., *Virgus*) and Mycetopodidae (e.g., *Lamproscapha*, Haag, 2012). The advantages of this shell morphology are unknown, but theories have ranged from reduced susceptibility to stressful environmental factors associated with high-flow events to having an optimal ratio shell size and thickness to reduce sinking in or enhance movements through softer substrates (Bailey and Green, 1988). It is worth noting that most taxa exhibiting a lance-like shell morphology are associated with soft-substrate habitats. The ubiquity of these

habitats in large, Coastal Plain rivers like many of the ones sampled in this study may explain the high degree of morphological convergence as well as phenotypic plasticity.

Results of my study highlight the fact that many phylogenetic relationships within Unionidae and other freshwater mussel groups remain unresolved and that taxonomic revision is needed for many groups. My four loci concatenated phylogenetic reconstructions suggests that *E. lanceolata* is not a true *Elliptio* but forms a clade sister to the Spiny mussel genus *Parvaspina*. Additionally, genetic data from across this species' range reveal low levels of genetic diversity that may indicate that this threatened species may be susceptible to inbreeding depression. Future status assessments should take genetic diversity data into consideration. My results also suggest that five currently recognized *Elliptio* taxa (*E. ahenea*, *E. angustata*, *E. folliculata*, *E. producta* and *E. shepardiana*) should be considered a single taxon (*E. angustata*) that, along with *E. fisheriana* groups within the "core"-*Elliptio* clade. This change has the potential to significantly change state and drainage-level management plans because *E. ahenea* has been petitioned for protection under the ESA and *E. shepardiana* and *E. folliculata* are considered at-risk taxa by several states along the Atlantic Slope. Conservation plans for *E. fisheriana* should consider this taxon as being comprised of three geographically distinct demes that may warrant consideration as individual management units. However, future research should examine whether life history, morphological or fine-scale genetic (e.g., microsatellite) differences support this consideration.

This study lays the groundwork for future *Elliptio* conservation genetics research. Future phylogenetic analysis should examine species boundaries in other *Elliptio* taxa to provide a basis for comparing taxonomic and genetic differences among taxa. More sensitive molecular methods including as next-generation sequencing or microsatellites may yet reveal additional structuring

within these taxa. Additionally, research is needed to better understand the mechanisms shaping *Elliptio* ecophenotypes and to understand the degree to which morphologies vary within and across drainages in order to help field biologists sort animals during monitoring surveys. Finally, genetic data will likely play an important role in future freshwater mussel monitoring and status assessments as they provide an objective way to assess species boundaries as well as provide critical information about genetic variability and deeper taxonomic relationships among freshwater mussel taxa.

References

Alderman, J., 2003. Status and Distribution of *Fusconaia masonii* and *Elliptio lanceolata* in Virginia. USFWS Grant Agreement:1148-401 81-99 G 113: 118pp.

Bandelt, H., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. and Evol.* 16, 37–48.

Bailey, R. C., Green, R. H., 1988. Within-basin variation in the shell morphology and growth rate of a freshwater mussel. *Can. J. Zool.* 66, 1704-1708.

Bogan A.E., 1996. *Elliptio ahenea*: *The IUCN Red List of Threatened Species 1996*. http://www.iucnredlist.org/species/7637/12838175. 29, March 2020.

Bogan A.E., 1996. *Elliptio shepardiana: The IUCN Red List of Threatened Species 1996.* https://www.iucnredlist.org/species/7650/12839800.29, March 2020

Ashton, M., Bogan, A.E., 2016. Manual of the Freshwater Bivalves of Maryland: *Maryland Department of Natural Resources*, Annapolis, Maryland.

Alderman, J., Bogan, A.E., 2008. Workbook and key to the freshwater bivalves of South Carolina: Revised Second Edition. *US Forest Service,* Columbia, SC.

Bogan, A.E., Levine, J., Raley, M., 2009. Determination of the systematic position and relationship of the lanceolate *Elliptio* complex (Mollusca:Bivalvia: Unionidae) from six river basins in Virginia. *Report to Virginia Department of Game and Inland Fisheries, Richmond, VA.*

Campbell, D.C., Lydeard, C., 2012. The Genera of *Pleurobemini* (Bivalvia: Unionidae: Ambleminae). *Am. Malacol. Bull*. 30, 19-38.

Campbell, D. C., Johnson, P. D., Williams, J. D., Rindsberg, A. K., Serb, J. M., Small, K. K., Lydeard, C., 2008. Identification of 'extinct' freshwater mussel species using DNA barcoding. *Mol.Ecol.Resour.* 8, 711-724.

Campbell., D.C., Serb, J. M., Buhay, J.E., Row, K. J., Minton, R. L., Lydeard, C., 2005. Phylogeny of North American Amblemines (Bivalvia, Unionida): prodigious polyphyly proves pervasive across genera. *Invertebr. Biol.* 124, 131-164.

Chivian, E., 2002. Biodiversity: its importance to human health. *Center for Health and the Global Environment, Harvard Medical School, Cambridge, MA*.

Clement, M., Snell, Q., Walker, P., Posada, D., Crandall, K. 2002. TCS: estimating gene genealogies. *Proc 16th Int Parallel Distrib Process Symp* 2. 184.

Darriba, D., Taboada, G. L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods*. 9, 772.

Davis, G.M., 1984. Genetic relationships among some North American Unionidae (Bivalvia): sibling species, convergence, and cladistic relationships. *Malacologia*. 25, 629–648.

Davis, G. M., Heard, W. H., Fuller, S. L. H., Hesterman, C., 1981. Molecular genetics and speciation in *Elliptio* and its relationships to other taxa of North American Unionidae (Bivalvia). *Biol. J. Linn. Soc.* 15, 131–150.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Sullivan, C. A. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev.* 81, 163-182.

Duffy, J. E., Godwin, C. M., & Cardinale, B. J., 2017. Biodiversity effects in the wild are common and as strong as key drivers of productivity. *Nature*, 549, 261-264.

Haag, W. R., Williams, J. D., 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. *Hydrobiologia*. 735, 45-60.

Hoang, W. R., Williams, J. D., 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. *Hydrobiologia*. 735, 45-60.

Horton, J.W., Zullo, V.A., 1991 *The Geology of the Carolinas: Carolina Geological Society Fiftieth Anniversary Volume*:Univ. of Tennessee Press, Knoxville, TN.

Inoue, K., Hayes, D. M., Harris, J. L., Johnson, N. A., Morrison, C. L., Eackles, M. S., ... Randklev, C. R., 2018. The Pleurobemini (Bivalvia: Unionida) revisited: molecular species delineation using a mitochondrial DNA gene reveals multiple conspecifics and undescribed species. *Invertebr.Syst*. 32, 689-702.

Isbell, F., Gonzalez, A., Loreau, M., Cowles, J., Díaz, S., Hector, A., Turnbull, L. A., Thompson P.L., Larigauderie A., 2017. Linking the influence and dependence of people on biodiversity across scales. *Nature*. *546*, 65-72.

Johnson, R., 1970. The systematics and zoogeography of the unionidae (Mollusca: Bivalvia*)* of the Southern Atlantic Slope Region. *Bull. of the Mus. Comp. Zool.* 140, 263–450.

Katoh, K., Standley, D.M., 2013., MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. and Evol.* 30, 772-780.

King, T., Eackles M., Gjetvaj B., Hoeh, W., 1999. Intraspecific phylogeography of *Lasmigona subviridis* (Bivalvia: Unionidae): conservation implications of range discontinuity. *Mol.Ecol.* 8, 65–78.

Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870-1874.

Lane, T. W., Hallerman, E. M., Jones, J. W., 2019. Population genetic assessment of two critically endangered freshwater mussel species, Tennessee bean *Venustaconcha trabalis* and Cumberland bean *Venustaconcha troostensis*. *Conserv. Genet.* 20, 759-779.

Maddison, W. P., Knowles, L. L., 2006. Inferring phylogeny despite incomplete lineage sorting. *Syst. Biol*. 55, 21-30.

Moore, R. H., Coney, C. C., Creitz, M. R., 1982. Comparisons of morphometric and soft anatomy characters between topotypic populations of *Elliptio lanceolata* and *E. angustata*. *Am. Malacol. Bull.* 1, 95.

Nguyen, L. T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Mol. Biol. Evol*. 32:268-274.

Perkins, M. A., Johnson, N. A., Gangloff, M. M., 2017. Molecular systematics of the critically endangered North American spinymussels (Unionidae: Elliptio and Pleurobema) and description of Parvaspina gen. nov. *Conserv. Genet*. 18, 745-757.

Rambaut, A., 2018. Version FigTree. 1.4. 4.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., Suchard, M. A., 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol*. 67, 901.

Roe, K. J., Hartfield, P. D., Lydeard, C., 2001. Phylogeographic analysis of the threatened and endangered superconglutinate-producing mussels of the genus *Lampsilis* (Bivalvia: Unionidae). *Mol.Ecol*. 10, 2225-2234.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., ... Huelsenbeck, J. P., 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol*. 61, 539-542.

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* 34, 3299-3302.

Serb, J. M., Buhay, J. E., Lydeard, C., 2003. Molecular systematics of the North American freshwater bivalve genus *Quadrula* (Unionidae:Ambleminae) based on mitochondrial ND1 sequences. *Mol. Phylogenet. Evol*. 28, 1-11.

Smith, C.H., Johnson, N.A., Inoue, K., Doyle, R. D., Randkley, C.R., 2019. Integrative taxonomy reveals a new species of freshwater mussel, *Potamilus strekersoni* sp. nov. (Bivalvia: Unionidae): implications for conservation and management. *Syst. and Biodivers*. 17, 331-348.

Smith, C.H., Johnson, N.A., Pfeiffer, J.M., Gangloff M. M., 2018. Molecular and morphological data reveal non-monophyly and speciation in imperiled freshwater mussels (*Anodontoides* and *Strophitus*). *Mol.Phylogenet. Evol*. 119, 50-62.

Therriault, T.W., Docker, M.F., Orlova, M.I., Heath, D.D., MacIssac, H.J., 2004. Molecular resolution of the family Dreissdenidae (Mollusca: Bivalvia) with emphasis on Ponto-Caspian species, including first report of *Mytilopsis leucophaeata* in the Black Sea basin. *Mol. Phylogenet. Evol*. 30, 479-489.

Tickner, D., Opperman, J. J., Abell, R., Acreman, M., Arthington, A. H., Bunn, S. E., ... Young, L., 2020. Bending the curve of global freshwater biodiversity loss: an emergency recovery plan. *BioScience*. 70, 330-342.

Turgeon, D. D., Quinn, Jr J. F., Bogan, A. E., Coan, E. V, Hochberg, F. G., Lyons, W. G., Mikkelsen, P. M., Neves, R. J., Roper, C. F. E., Rosenberg, G., 1998. Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks, 2nd edition: *Am. Fish. Soc. Spe.Publ 26*. Bethesda, MD.

U.S. Fish and Wildlife Service, 2016. Species Status Assessment Report for the Yellow Lance (*Elliptio lanceolata*): *USFWS*, Atlanta, GA.

Van der Plas, F., 2019. Biodiversity and ecosystem functioning in naturally assembled communities. *Biol. Rev*. 94, 1220-1245.

Vaughn, C.C., Hakenkamp, C.C., 2001. The functional role of burrowing bivalves in freshwater ecosystems. *Freshw. Biol*. 46, 1431-1446.

Williams, J.D., Bogan, A.E., Butler, R. S., Cummings, K.S., Garner, J. T., Harris, J. L., ...Watters, G.T., 2017. A revised list of the freshwater mussels (Mollusca: Bivalvia. Unionida) of the United States and Canada. *Freshw. Mollusk Biol. Conserv*. 20, 33-59.

Wolfe, D. A., 1984. *Elliptio lanceolata*: Was Issac Lea's original description proper after all? *Am. Malacol.Bull*. 3, 94.

Tables and Figures

Taxon	State	Waterbody	Basin	I.D	CO1	ND1	ITS-1	28s
ahenea	FL	Suwannee	Suwannee	EaheSuw81	X	X	X	X
ahenea	FL	Suwannee	Suwannee	EaheSuw82	X	X	X	X
ahenea	FL	Fisheating Creek	Everglades	EspiEve009	X	X	X	EaheSuw82
ahenea	FL	Fisheating Creek	Everglades	EspiEve012	X	X	X	EaheSuw82
ahenea	FL	Arbuckle Creek	Everglades	EspiEve018	X	X	X	EaheSuw82
ahenea	FL	Redwater Lake	St. Johns	EhazStJ001	X	X	X	EaheSuw82
ahenea	FL	Redwater Lake	St. Johns	EhazStJ005	X	X	X	EaheSuw82
ahenea	FL	St. Marys River	St. Marys	EhazStm011	X	X	X	EaheSuw82
ahenea	FL	St. Marys River	St. Marys	EhazStm012	X	X	X	EaheSuw82
ahenea	FL	St. Marys River	St. Marys	EhazStm013	X	X	X	EaheSuw82
ahenea	FL	Suwannee River	Suwannee	EspiSuw042	X	X	X	EaheSuw82
ahenea	FL	Suwannee River	Suwannee	EspiSuw043	X	X	X	EaheSuw82
ahenea	FL	Suwannee River	Suwannee	EspiSuw044	X	X	X	EaheSuw82
ahenea	FL	Unnamed tributary (New River)	Suwannee	EhazSuw014	X	X	X	EaheSuw82
ahenea	FL	Unnamed tributary (New River)	Suwannee	EaheSuw114	X	X	X	EaheSuw91
ahenea	FL	Suwannee River	Suwannee	EaheSuw120	X	X	X	EaheSuw92
ahenea	FL	Ocklawaha River	St. Johns	EaheStJ123	X	X	X	EaheSuw93
angustata	NC	Big Creek	Waccamaw	EfisWac009	X	X	X	EfshWac059
angustata	NC	Big Creek	Waccamaw	EfisWac004	X	X	X	EfshWac059
angustata	NC	Big Creek	Waccamaw	EfisWac005	X	X	X	EfshWac059
angustata	NC	Big Creek	Waccamaw	EfisWac007	X	X	X	EfshWac059
angustata	NC	Big Creek	Waccamaw	EfisWac008	X	X	X	EfshWac059
angustata	SC	Catawba River	Santee-Cooper	EangSan2902	X	X	X	X
angustata	SC	Catawba River	Santee-Cooper	EangSan2901	X	X	X	X
angustata	SC	Congaree River	Santee-Cooper	EangSan7512	X	X	X	X

angustata	SC	Congaree River	Santee-Cooper	EangSan7511	X	X	N/A	N/A
angustata	SC	Santee River	Santee	EfisSan001	X	X	X	EangSan2902
angustata	SC	Santee River	Santee	EangSan002	X	X	X	EangSan2902
angustata	SC	Santee River	Santee	EfisSan003	X	X	X	EangSan2902
fisheriana	MD	Zekiah Swamp Run	Potomac	EfisPot016	X	X	X	EfshPot123
fisheriana	MD	Zekiah Swamp Run	Potomac	EfisPot017	X	X	X	EfshPot123
fisheriana	MD	Unicorn Branch	Chester River	EfisChe018	X	X	X	EfshChe023
fisheriana	MD	Unicorn Branch	Chester River	EfisChe020	X	X	X	EfshChe023
fisheriana	MD	Unicorn Branch	Chester River	EfisChe022	X	X	X	EfshChe023
fisheriana	MD	Unicorn Branch	Chester River	EfisChe025	X	X	X	EfshChe023
fisheriana	MD	Potomac River	Potomac	EfshPot116	X	X	X	X
fisheriana	MD	Potomac River	Potomac	EfshPot123	X	X	X	X
fisheriana	MD	Potomac River	Potomac	EfshPot028	X	X	N/A	N/A
fisheriana	MD	Unicorn Branch	Chester	EfshChe023	X	X	X	X
fisheriana	MD	Unicorn Branch	Chester	EfshChe028	X	X	X	X
fisheriana	NC	Waccamaw River	Pee Dee	EfshWac059	X	X	X	X
fisheriana	NC	Waccamaw River	Pee Dee	EfshWac079	X	X	X	X
fisheriana	NC	Waccamaw River	Pee Dee	EfshWac081	X	X	X	X
fisheriana	NC	Dan River	Roanoke	EfshRoa001	X	X	X	X
fisheriana	NC	Roanoke	Roanoke	EfshRoa012	X	X	N/A	N/A
fisheriana	NC	Roanoke	Roanoke	EfshRoa011	X	X	N/A	N/A
fisheriana	NC	Roanoke	Roanoke	EfshRoa004	X	X	N/A	N/A
fisheriana	NC	Congaree	Cooper	EfshSan032	X	X	N/A	N/A
fisheriana	NC	Congaree	Cooper	EfshSan031	X	X	N/A	N/A
fisheriana	NC	Roanoke River	Roanoke	EfisRoa014	X	X	X	EfshPot123
folliculata	NC	Lake Waccamaw	Pee Dee	EfolWac001	X	X	X	X
folliculata	NC	Lake Waccamaw	Pee Dee	EfolWac007	X	X	X	X
folliculata	NC	Lake Waccamaw	Pee Dee	EfolWac009	X	X	X	EfolWac007

folliculata	NC	Lake Waccamaw	Pee Dee	EfolWac022	X	X	X	X
folliculata	NC	Lake Waccamaw	Pee Dee	EfolWac005	X	X	X	X
lanceolata	NC	Hawlings	Patuxent	ElanPat059	X	X	X	X
lanceolata	MD	Hawlings	Patuxent	ElanPat075	X	X	X	X
lanceolata	MD	Hawlings	Patuxent	ElanPat077	X	X	X	X
lanceolata	MD	Hawlings	Patuxent	ElanPat089	X	X	X	ElanPat059
lanceolata	MD	Hawlings	Patuxent	ElanPat086	X	X	X	X
lanceolata	MD	Hawlings	Patuxent	ElanPat065	X	X	X	ElanPat059
lanceolata	NC	Tar River	Pamlico	ElanPam107	X	X	X	X
lanceolata	NC	Tar River	Pamlico	ElanPam120	X	X	X	X
lanceolata	NC	Fishing Creek	Pamlico	ElanPam128	X	X	X	X
lanceolata	NC	Fishing Creek	Pamlico	ElanPam108	X	X	X	X
lanceolata	NC	Fishing Creek	Pamlico	ElanPam126	X	X	X	X
lanceolata	NC	Swift Creek	Pamlico	ElanPam005	X	X	X	ElanPam126
lanceolata	NC	Swift Creek	Pamlico	ElanPam006	X	X	X	ElanPam126
lanceolata	NC	Swift Creek	Pamlico	ElanPam008	X	X	X	ElanPam126
lanceolata	NC	Swift Creek	Pamlico	ElanPam009	X	X	X	ElanPam126
lanceolata	NC	Swift Creek	Pamlico	ElanPam010	X	X	X	ElanPam126
lanceolata	NC	Swift Creek	Pamlico	ElanPam011	X	X	X	ElanPam126
lanceolata	NC	Swift Creek	Pamlico	ElanPam012	X	X	X	ElanPam126
lanceolata	NC	Swift Creek	Pamlico	ElanPam013	X	X	X	ElanPam126
lanceolata	NC	Swift Creek	Pamlico	ElanPam014	X	X	X	ElanPam126
lanceolata	NC	Carter Run	Rappahannock	ElanRap001	X	X	X	ElanPam126
lanceolata	VA	Carter Run	Rappahannock	ElanRap002	X	X	X	ElanPam126
lanceolata	VA	Carter Run	Rappahannock	ElanRap003	X	X	X	ElanPam126
lanceolata	VA	Carter Run	Rappahannock	ElanRap004	X	X	X	ElanPam126
producta	GA	Savannah River	Savannah	EproSav002	X	X	X	EproSav021
producta	GA	Savannah River	Savannah	EproSav003	X	X	X	EproSav021
producta	GA	Ogeechee River	Ogeechee	EproOge010	X	X	X	EproSav021

producta	GA	Oconee River	Altamaha	EproAlt011	X	X	X	EproSav021
producta	GA	Oconee River	Altamaha	EproAlt012	X	X	X	EproSav021
producta	GA	Oconee River	Altamaha	EproAlt013	X	X	X	EproSav021
producta	GA	Buckhead Creek	Ogeechee	EproOge016	X	X	X	EproSav021
producta	GA	Ogeechee River	Ogeechee	EangOge003	X	X	X	EproSav021
producta	GA	Little Ohoopee River	Altamaha	EangAlt004	X	X	X	EproSav021
producta	GA	Little Ohoopee River	Altamaha	EangAlt005	X	X	X	EproSav021
producta	GA	Little Ohoopee River	Altamaha	EangAlt006	X	X	X	EproSav021
producta	GA	Little Ohoopee River	Altamaha	EangAlt007	X	X	X	EproSav021
producta	GA	Little Ohoopee River	Altamaha	EangAlt008	X	X	X	EproSav025
producta	SC	Mountain Creek	Savannah	EproSav021	X	X	X	X
producta	SC	Savannah River	Savannah	EproSav029	X	X	X	EproSav021
producta	SC	Savannah River	Savannah	EproSav030	X	X	X	EproSav021
shepardiana	GA	Ohoopee River	Altamaha	EsheAlt112	X	X	X	X
shepardiana	GA	Ohoopee River	Altamaha	EsheAlt062	X	X	X	X
shepardiana	GA	Altamaha River	Altamaha	EsheAlt001	X	X	X	EsheAlt112
shepardiana	GA	Altamaha River	Altamaha	EsheAlt002	X	X	X	EsheAlt112
shepardiana	GA	Altamaha River	Altamaha	EsheAlt006	X	X	X	EsheAlt112

Table 1. Gangloff and Johnson unpublished specimen Collection and locality Information. X indicates included sequence in analysis. Sequence name in 28s column refers to sequence used to complete four loci dataset.

Species	n	State	Waterbodies	Drainage	County
Eurynaia dilatata	3	TN	Clinch River (Cleveland Islands)	Tennessee	Russell
Eurynaia dilatata (28s only)	1	NC	Hiawassee River	Tennessee	Cherokee
Pleuronaia barnesiana	2	VA	Clinch River (Cleveland Islands)	Tennessee	Russell
*Plethobasus cyphyus***	2	TN	Clinch River (Clinchport)	Tennessee	Scott
*Hemistena lata***	2	TN	Clinch River (Kyles Ford and Frost Ford)	Tennessee	Hancock
Parvaspina steinstansana	2	NC	Fishing Creek	Pamlico	Halifax
*Parvaspina collina***	2	VA	James River	James	Albemarle
Elliptio arca	2	MS	Tombigbee	Mobile	Monroe
*Elliptio arctata***	2	AL	Clear Creek	Mobile	Fayette
*Elliptio crassidens*** (28s only)	1	AL	Cahaba River	Mobile	Shelby

Table 2. Outgroup Specimen Collection and Locality Information. Chimeric specimens are highlighted with ** signifying sequences with multiple vouchered specimen (source of multiple specimen can be matched with (Table 3)

Species	CO1	ND1	ITS1	28s	References
Amblema plicata	MK044903	MK045053	MK036154	MK036070	Smith et al. 2019
Amblema plicata	MK044904	MK045054	MK036155	MK036071	Smith et al. 2019
Quadrula Quadrula	MH633643	MH633595	MH362613	MK036133	Smith et al. 2019
Regina ebenus	MK044965	MK045116	MK0360216	MK036134	Johnson et al. 2018
Regina ebenus	MK044966	MK045117	MK036217	MK036135	Smith et al. 2019
Plectomerus dombeyanus	MK044938	MK045089	MK036189	MK036106	Smith et al. 2019
Plectomerus dombeyanus	MK044939	MK04590	MK036190	MK036107	Smith et al. 2019
Pleurobema clava	MF962113	AY613802	DQ383449	N/A	Campbell et al. 2005, 2008. Inoue et al. 2018
Fusconaia flava	MH133599	MH133759	MH13388	MK001829	Pieri et al. 2018
Elliptio crassidens	MH633634	MH633586	MH362521	Table 2	Johnson et al. 2018, Gangloff et al. unpubl data
Elliptio crassidens	MH633644	MH633596	MH362615	Table 2	Johnson et al. 2018, Gangloff et al. unpubl data

Elliptoideus sloatianus	KT285623	AY613790	KT285667	MK001834	Pfeiffer and Johnson 2015, Campbell et al. 2005, Pfeiffer, 2015
Hemistena lata	Table 2	Table 2	DQ383443	Table 2	Gangloff et al unpubl. Data, Campbell et al. 2008
Elliptio arctata	Table 2	Table 2	DQ383438	Table 2	Gangloff et al unpubl. Data, Campbell et al. 2008
Plethobasus cyphyus	Table 2	Table 2	DQ383445	Table 2	Gangloff et al unpubl. Data, Campbell et al. 2008
Elliptio spinosa	KU696961	KU696949	KU726538	N/A	Perkins et al. 2017
Parvaspina collina	MK044903	MK045053	MK036070	MK036070	Smith et al. 2019
Parvaspina collina	MK044904	MK045054	MK036071	MK036071	Smith et al. 2019

Table 3. GenBank sequences used to supplement gaps in four locus analysis. Chimeric symbols are represented by ** (multiple vouchered specimen for a single four loci alignment).

Locus	Origin	Forward	Reverse	Expected Product Size
CO1	Mitochondrial	GTTCCACAAATCATAAGGATATTGG	TACACCTCAGGGTGACCAAAAAACCA	700 bp
ND1	Mitochondrial	TGGCAGAAAAGTGCATCAGATTAAAC	TGGCAGAAAAGTGCATCAGATTAAAC	900 bp
ITS-1	Nuclear	AAAAAGCTTCCGTAGGTGAACCTGCGAGCTTGCTGCTGTCTTCATCG	AGCTTGCTGCTGTCTTCATCG	500 bp
28s	Nuclear	TCCGATAGCGCACAAGTACC	TTGCACGTCAGAATCGCTAC	600 bp

Table 4 primers used for phylogenetic analyses

		1	2	3	4	5	6	7	8	9	10
1	*Elliptio s.s*	-									
2	*E. lanceolata*	0.094	-								
3	*E. fisheriana*	0.061	0.082	-							
4	*E. shepardiana*	0.051	0.088	0.056	-						
5	*E. angustata*	0.050	0.085	0.054	0.006	-					
6	*E. producta*	0.050	0.087	0.056	0.002	0.006	-				
7	*E. ahenea*	0.050	0.086	0.055	0.002	0.005	0.002	-			
8	*E. folliculata*	0.049	0.084	0.053	0.007	0.006	0.007	0.006	-		
9	*Parvaspina*	0.083	0.082	0.083	0.074	0.072	0.073	0.073	0.072		
10	*Eurynaia dilatata*	0.064	0.087	0.072	0.063	0.061	0.063	0.062	0.060	0.078	-

Table 5. Inter-specific pairwise genetic distances of CO1 dataset. Pairwise genetic distances calculated using maximum composite likelihood

		1	2	3	4	5	6	7	8	9	10
1	*Elliptio s.s*	-									
2	*E. lanceolata*	0.124	-								
3	*E. fisheriana*	0.062	0.098	-							
4	*E. shepardiana*	0.062	0.117	0.056	-						
5	*E. angustata*	0.056	0.113	0.049	0.001	-					
6	*E. producta*	0.061	0.116	0.056	0.004	0.001	-				
7	*E. ahenea*	0.058	0.114	0.054	0.004	0.009	0.003	-			
8	*E. folliculata*	0.059	0.116	0.053	0.009	0.006	0.008	0.008	-		
9	*Parvaspina*	0.099	0.113	0.089	0.090	0.086	0.091	0.088	0.088		
10	*Eurynaia dilatata*	0.095	0.100	0.082	0.092	0.085	0.091	0.090	0.088	0.102	-

Table 6. Inter-specific pairwise genetic distances of ND1 dataset. Pairwise genetic distances calculated using maximum composite likelihood

		1	2	3	4	5	6	7	8	9	10
1	*Elliptio s.s*	-									
2	*E. lanceolata*	0.014	-								
3	*E. fisheriana*	0.033	0.025	-							
4	*E. shepardiana*	0.014	0.000	0.025	-						
5	*E. angustata*	0.021	0.009	0.020	0.009	-					
6	*E. producta*	0.015	0.001	0.025	0.001	0.010	-				
7	*E. ahenea*	0.015	0.001	0.025	0.001	0.010	0.002	-			
8	*E. folliculata*	0.021	0.006	0.031	0.006	0.014	0.007	0.008	-		
9	*Parvaspina*	0.037	0.029	0.032	0.029	0.031	0.029	0.030	0.035	-	
10	*Eurynaia dilatata*	0.017	0.003	0.028	0.003	0.012	0.004	0.005	0.009	0.032	-

Table 7. Inter-specific pairwise genetic distances of ITS-1 dataset. Pairwise genetic distances calculated using maximum composite likelihood

	1	2	3	4
1	North	-		
2	Dan	0.020	-	
3	South	0.028	0.035	-

Table 8. Inter-specific pairwise genetic distances of CO1 dataset based on genetic structuring *of E. fisheriana* across three distinct geographic regions (North, Dan and South).

	1	2	3	4
1	Rappahannock	-		
2	Patuxent	0.008	-	
3	Pamlico	0.010	0.006	-

Table 9. Inter-specific pairwise genetic distances of CO1 dataset based on genetic structuring *of E. lanceolata* across three distinct geographic regions (Rappahannock, Patuxent and Pamlico).

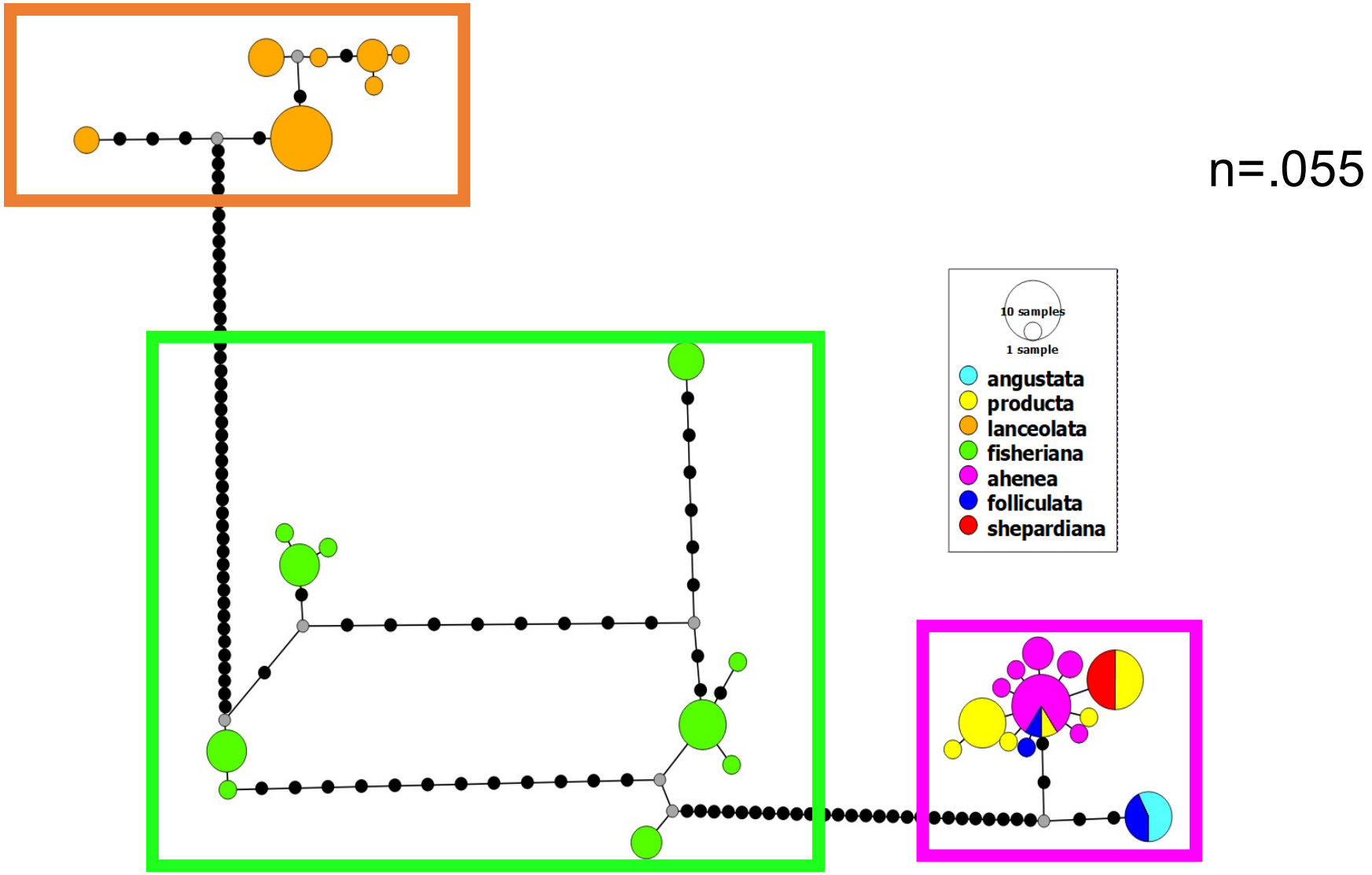

Fig 1. TCS Haplotype network of individual locus CO1 by species. Size of circle represents number of individuals sharing a given haplotype. Filled in circles at junctions represent implied or unsampled haplotypes. Numbers represent the number of nucleotide mutations. n= nucleotide diversity. Three squares represent three separate haplogroups (*E. lanceolata* = orange, *E. fisheriana* = green *E. angustata* = pink).

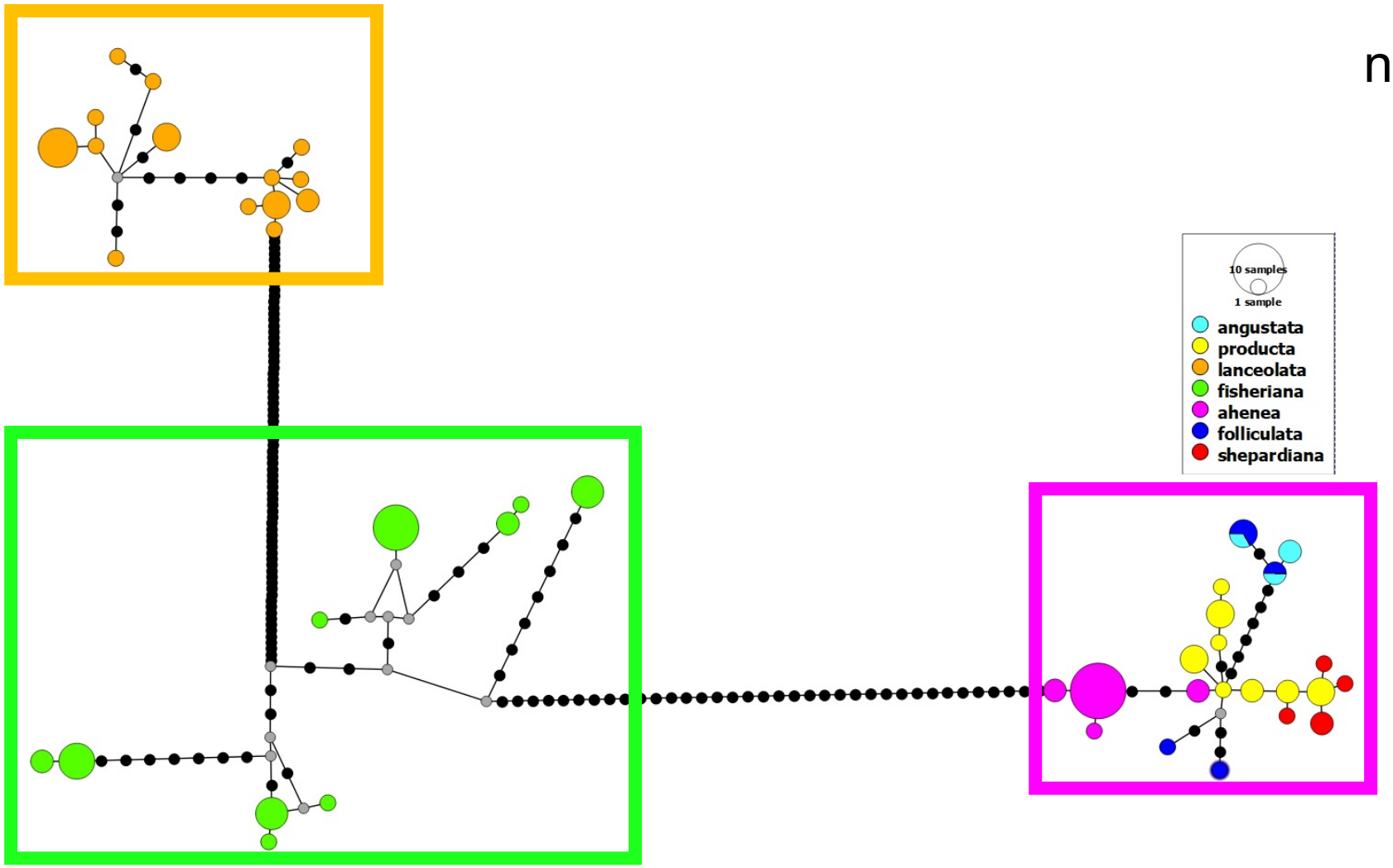

Fig 2. TCS Haplotype network of individual locus ND1 by species. Size of circle represents number of individuals sharing a given haplotype. Filled in circles at junctions represent implied or unsampled haplotypes. Numbers represent the number of nucleotide mutations. n= nucleotide diversity. Three squares represent three separate haplogroups (*E. lanceolata* = orange, *E. fisheriana* = green *E. angustata* = pink).

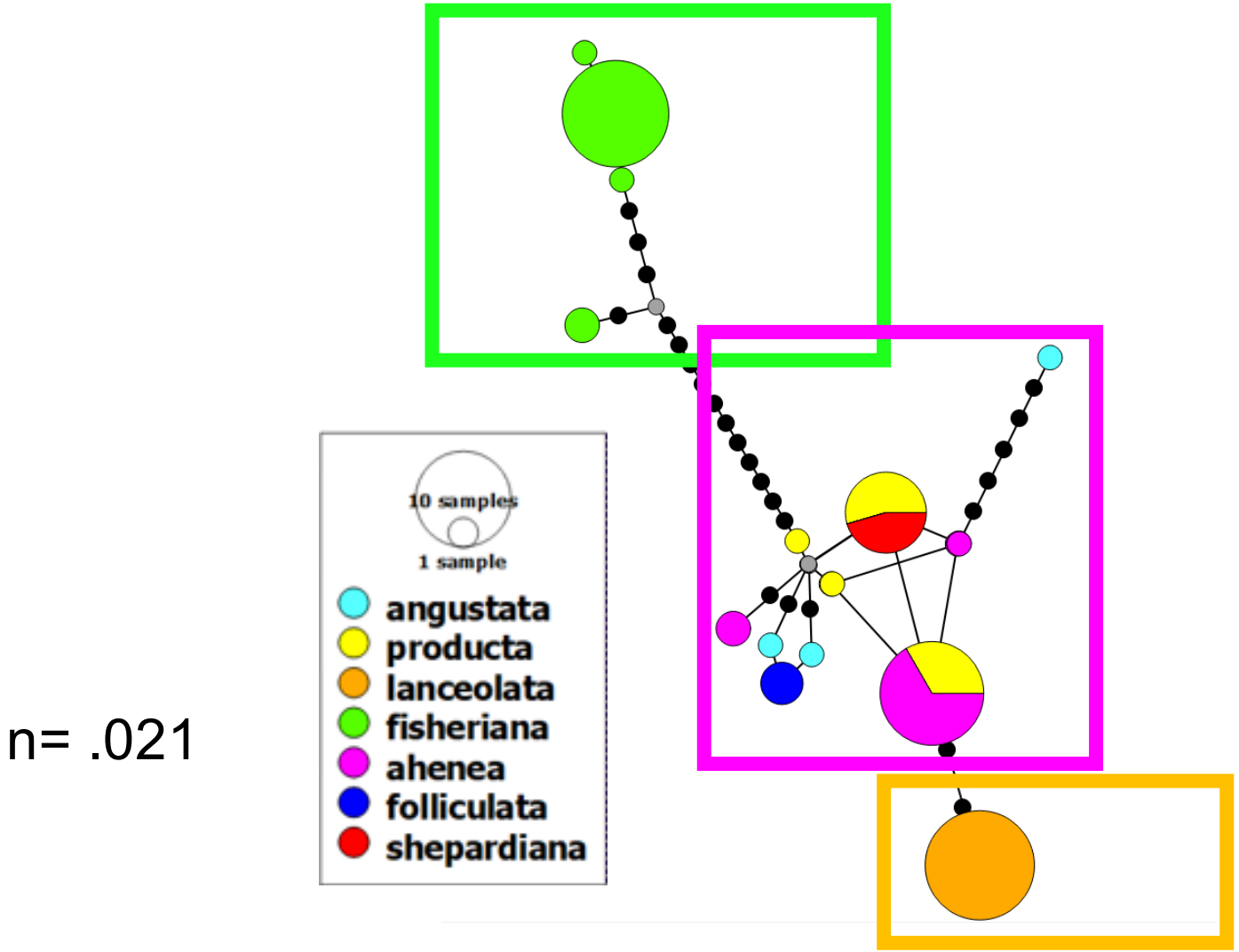

Fig 3. TCS Haplotype network of individual locus CO1 by species. Size of circle represents number of individuals sharing a given haplotype. Filled in circles at junctions represent implied or unsampled haplotypes. Numbers represent the number of nucleotide mutations. n= nucleotide diversity. Three squares represent three separate haplogroups (*E. lanceolata* = orange, *E. fisheriana* = green *E. angustata* = pink).

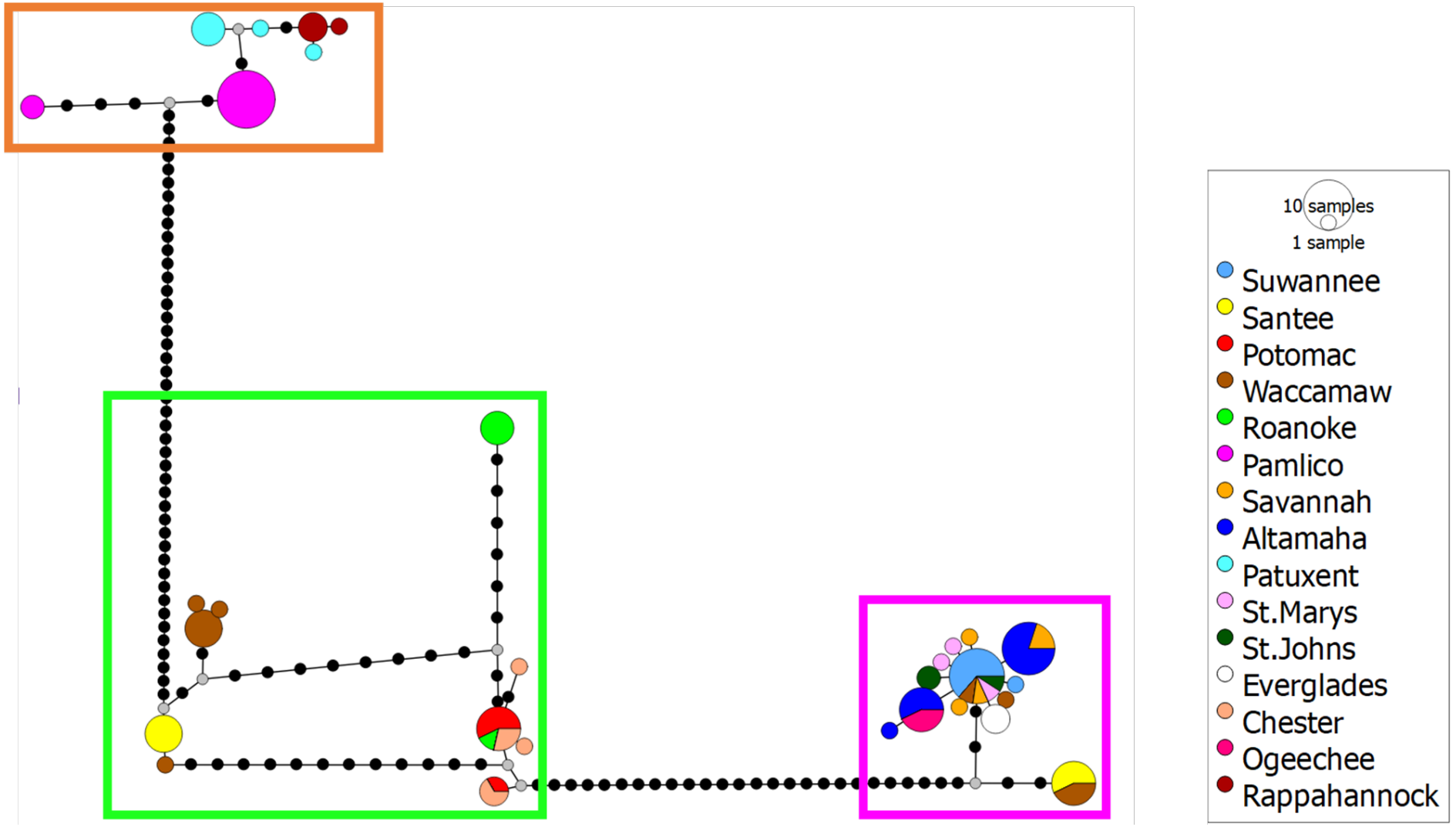

Fig 4. TCS Haplotype network of CO1 dataset coded by drainage. Size of circle represents number of individuals sharing a given haplotype. Filled in circles at junctions represent implied or unsampled haplotypes. Numbers represent the number of nucleotide mutations. Three squares represent three separate haplogroups (*E. lanceolata* = orange, *E. fisheriana* = green *E. angustata* = pink).

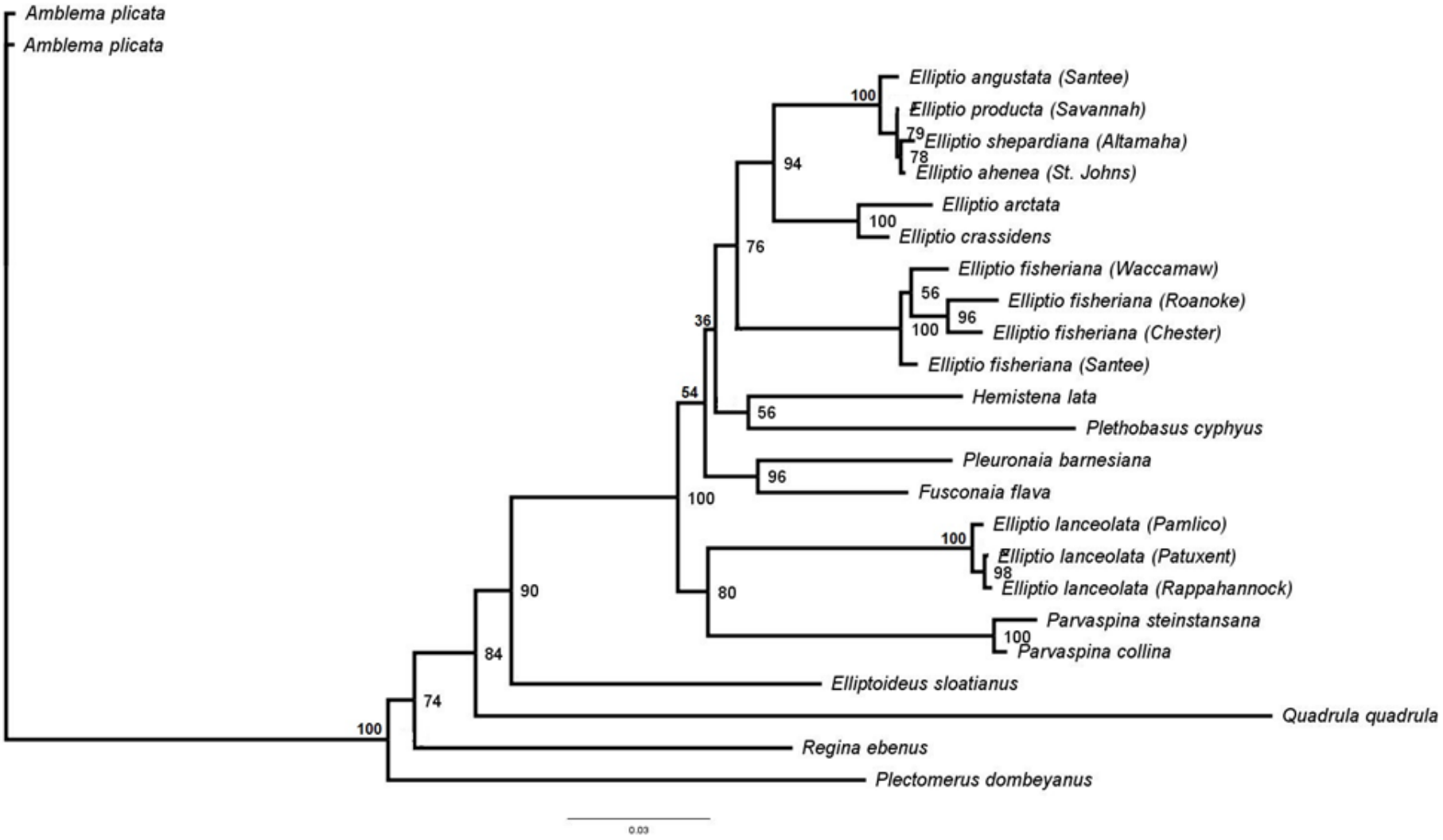

Fig 5. Phylogenetic reconstruction based on Maximum likelihood concatenated 4 loci dataset (CO1, ND1, ITS-1 and 28s). Node labels indicate Ultrafast bootstrap values.

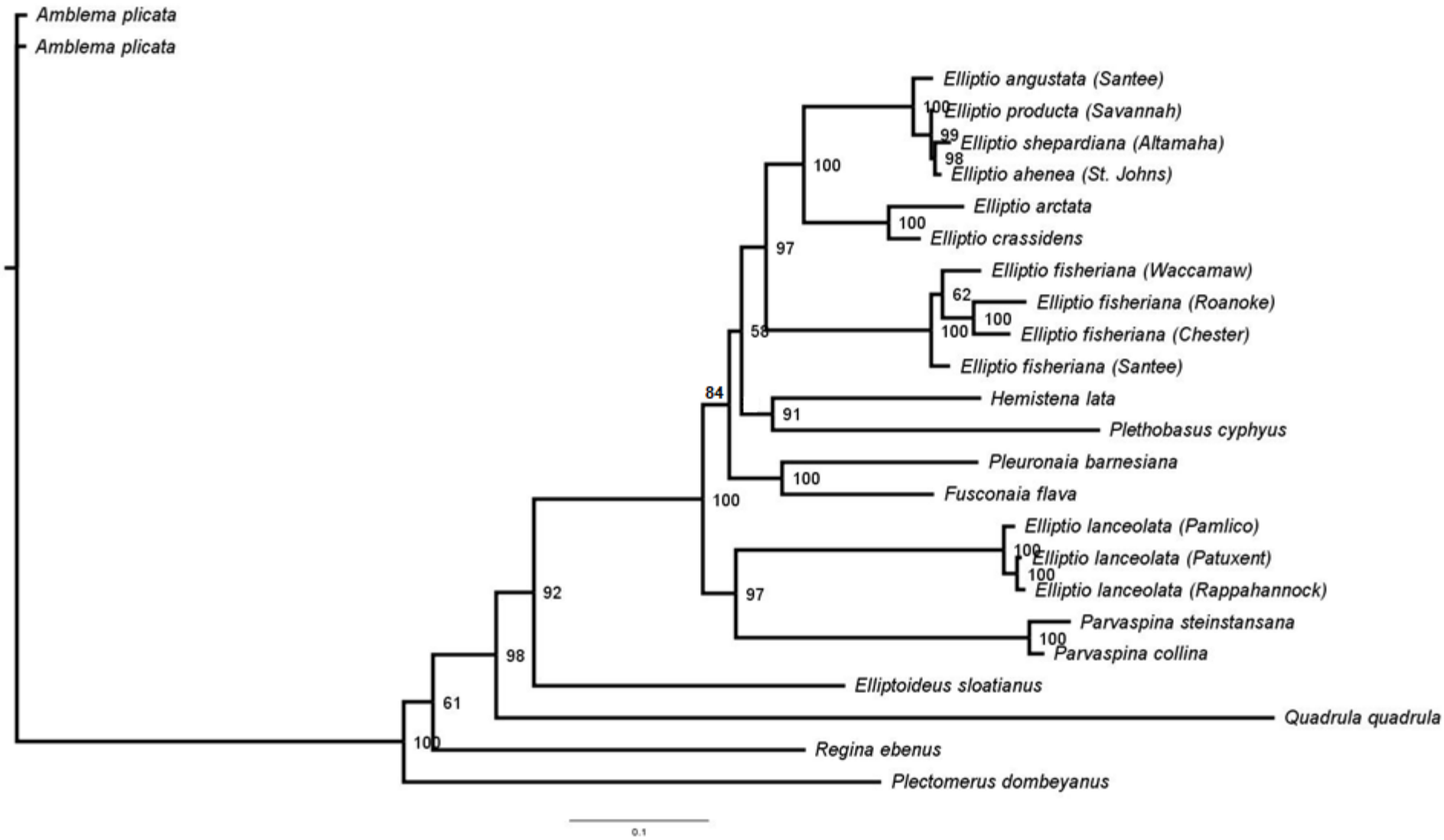

Fig 6. Phylogenetic reconstruction based on Bayesian Inference concatenated 4 loci dataset (CO1, ND1, ITS-1 and 28s). Node labels indicate posterior probability values.

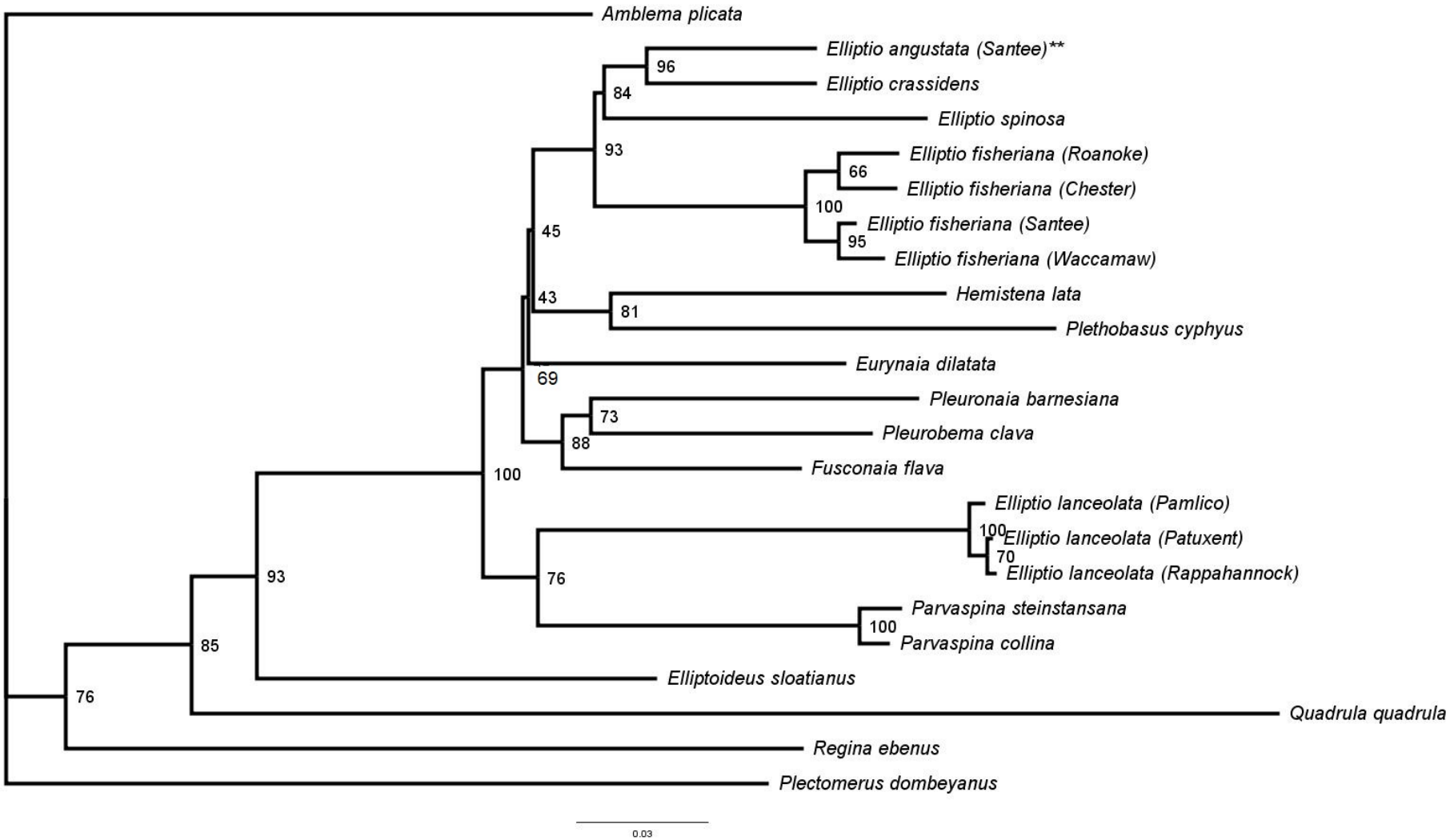

Fig 7. Phylogenetic reconstruction based on Maximum likelihood of the concatenated mtDNA dataset (CO1, ND1). Node labels indicate Ultrafast bootstrap values. ** indicates *E. angustata sensu lata* clade.

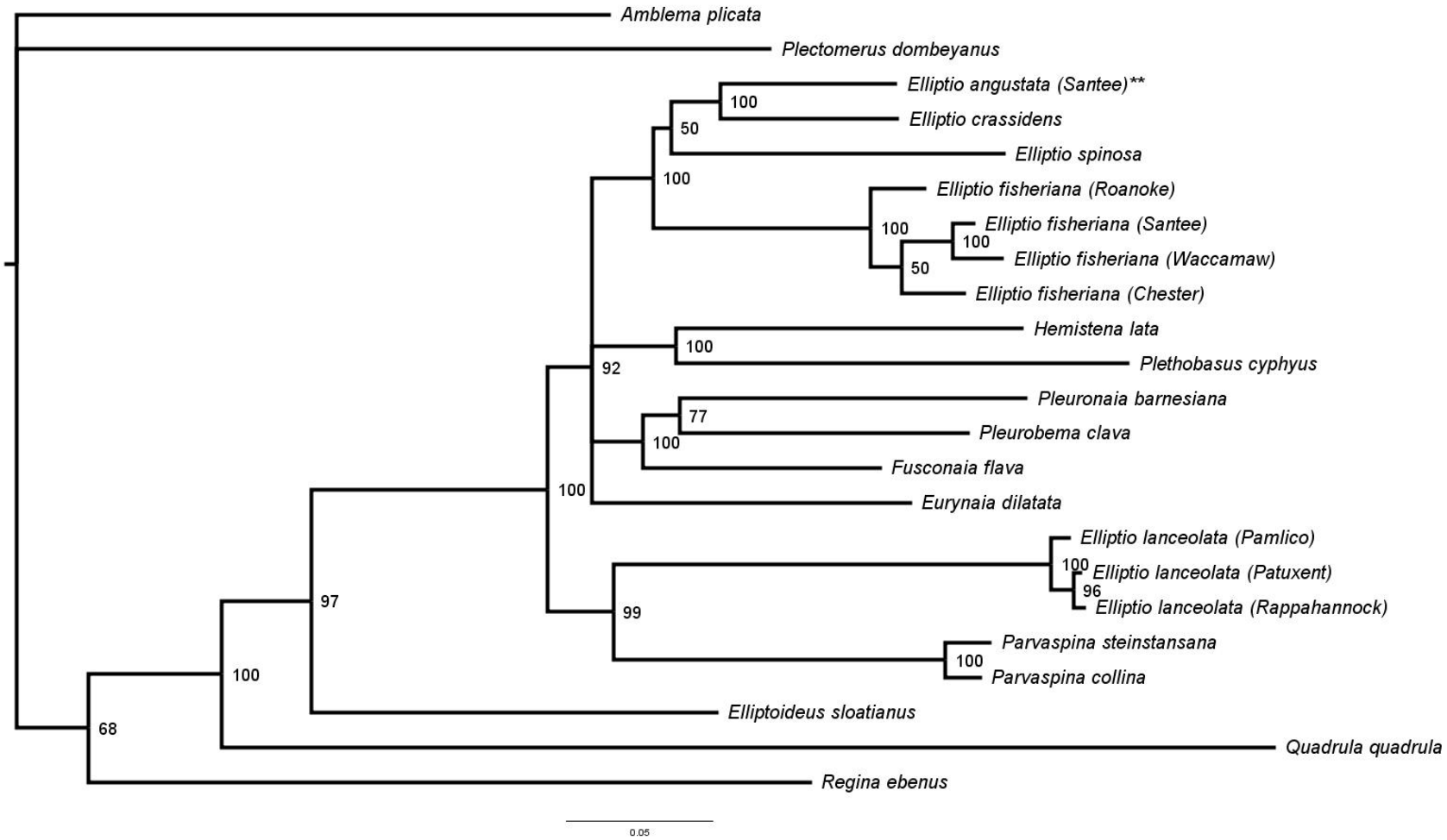

Fig 8. Phylogenetic reconstruction based on Bayesian Inference of the concatenated mtDNA dataset (CO1, ND1). Node labels indicate posterior probability values. ** indicates *E. angustata sensu lata* clade

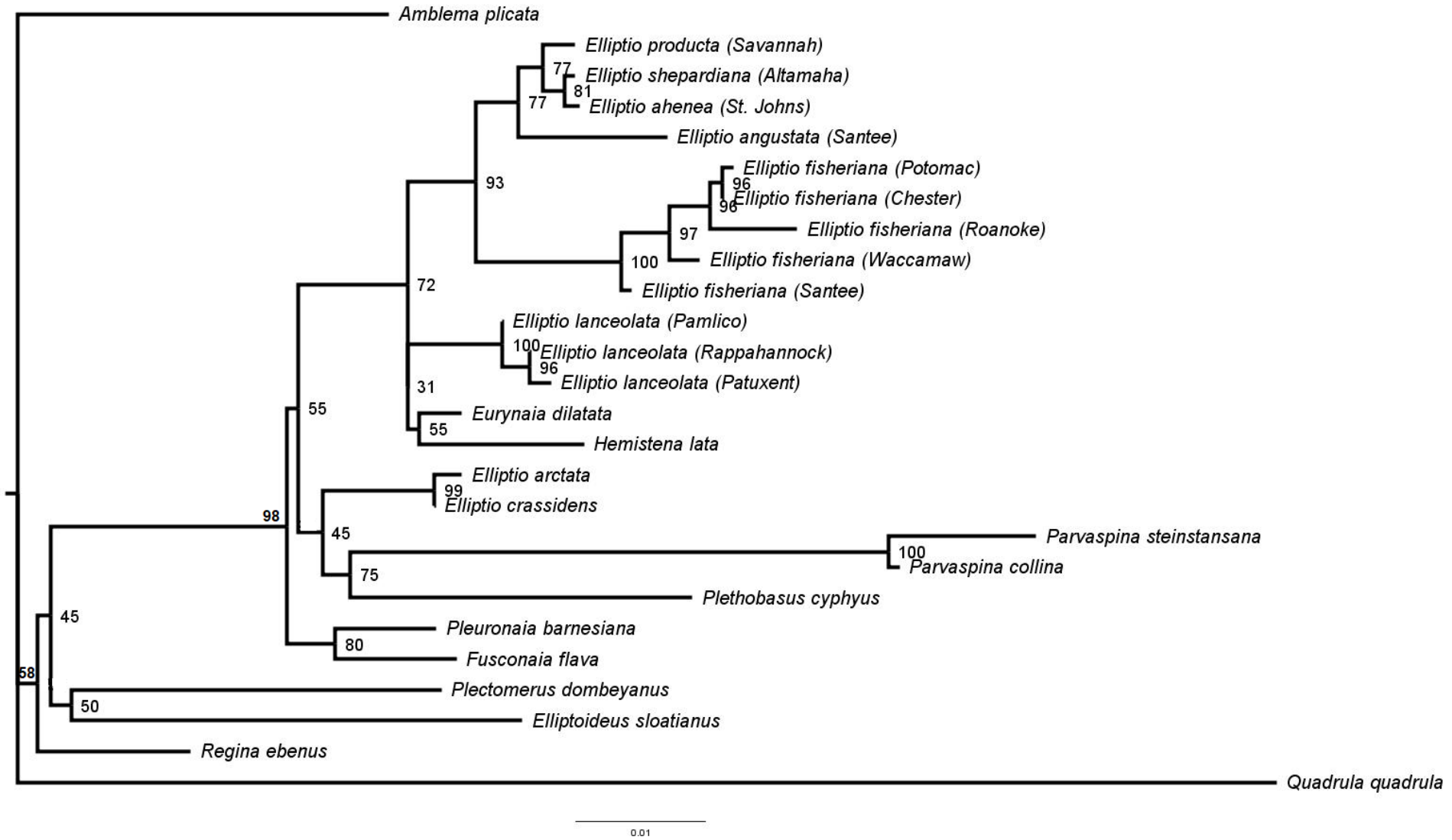

Fig 9. Phylogenetic reconstruction based on Maximum likelihood of the concatenated nDNA dataset (ITS1 and 28s). Node labels indicate Ultrafast bootstrap values.

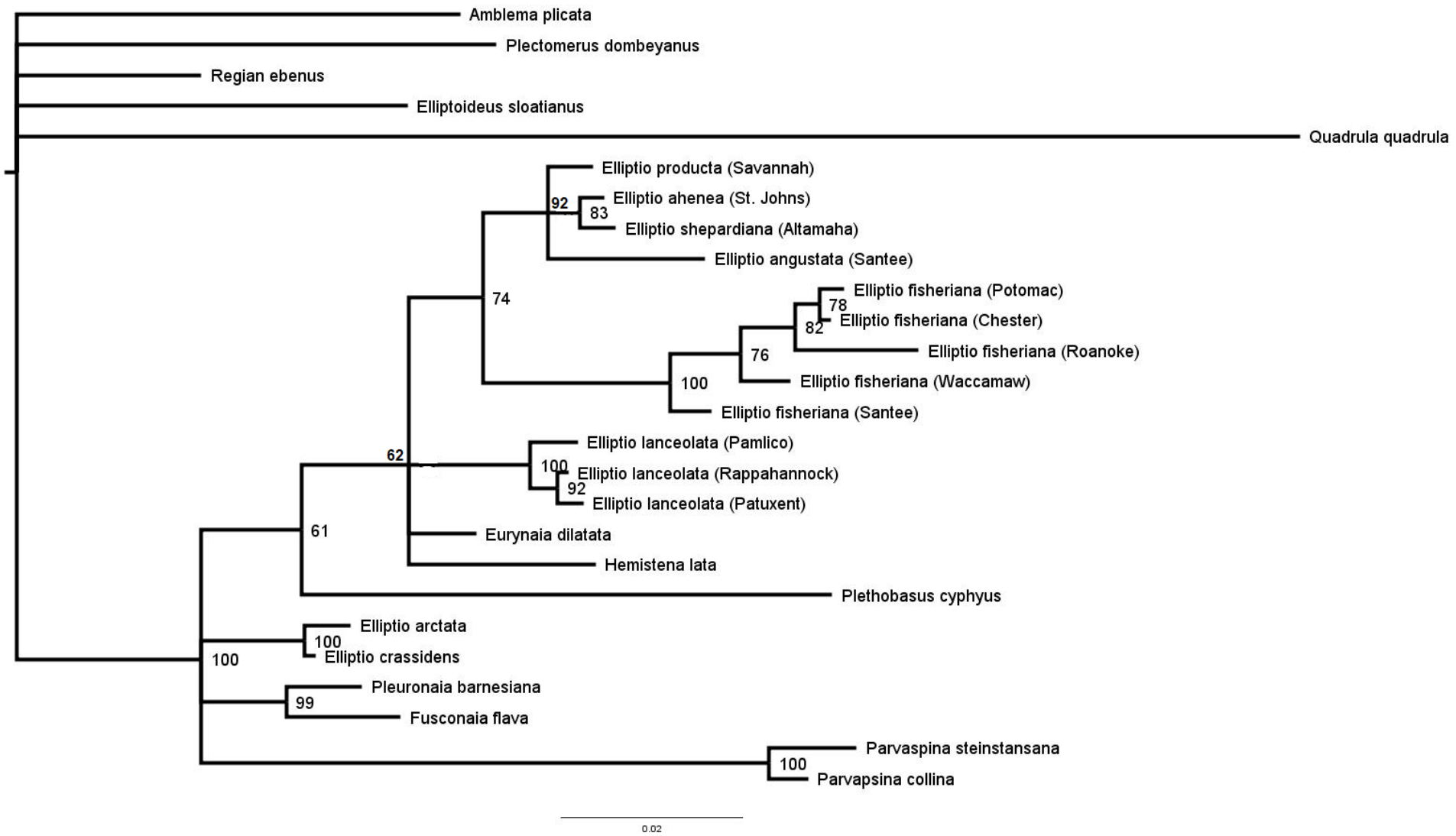

Fig 10. Phylogenetic reconstruction based on Bayesian Inference of the concatenated nDNA dataset (ITS1 and 28s). Node labels indicate posterior probability values.

Vita

Hans Robert Lohmeyer was born in Sacramento, California to Nancy and Norman Lohmeyer in 1992. He grew up in McLeansville, NC. He received his Bachelor of Science degree from the University of North Carolina at Asheville in 2014. He is currently employed by the North Carolina Wildlife Resources Commission as a Snail Aquaculturist Technician in Marion, NC. He currently resides in Asheville, NC with his wife.